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Effect of Dietary Vitamin A and Zinc on Egg Yield and Some Blood Parameters of Laying Hens

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Abstract: The purpose of the present study was to examine the effects of vitamin A and zinc interaction in laying hens fed on diets supplemented with two different levels of vitamin A (0, 10.000 IU/kg) in combination with five different levels of zinc (0, 25, 50, 100, 200 mg/kg) on egg yield, plasma and yolk concentration of zinc, and the levels of plasma triglyceride and phosphorus for 12 weeks.

Egg production, egg weight, feed intake, body weight and feed efficiency did not differ among the groups. Plasma and yolk zinc concentrations were affected only by zinc supplementation. An interaction between vitamin A and zinc had an effect and the independent effect of vitamin A supplementation increased the plasma triglyceride levels of laying hens. The level of phosphorus in plasma was altered by zinc supplementation and an interaction between zinc and vitamin A. Since the positive relationship between plasma zinc and egg production was observed ($r=0.279$ $p<0.01$), it is suggested that plasma zinc may be an indicator for the estimation of egg production or hen performance.

Key Words: zinc, vitamin A, egg production.

Yumurtacı Tavukların Yumurta Verimi ve Bazı Kan Parametreleri Üzerine Rasyondaki Vitamin A ve Çinkonun Etkisi

Özet: Bu çalışmanın amacı, yumurtacı tavukların iki farklı düzeyde vitamin A(0-10 000 IU/kg) ile kombine beş farklı düzeyde çinko (0,25,50,100,200 mg/kg) ilave edilmiş rasyonla 12 hafta beslenmesi durumunda vitamin A ve çinko etkileşiminin yumurta verimi, plazma ve yumurta sarısı çinko konsantrasyonu ile plazma trigliserit ve fosforu üzerine etkisini araştırmaktır.

Yumurta verimi, yumurta ağırlığı, yem tüketimi, canlı ağırlık ve yemden yararlanma açısından gruplar arasında farklılık yoktu. Plazma ve yumurta sarısı çinko konsantrasyonları sadece çinko ilavesinden etkilendi. Vitamin A ve çinko etkileşimi plazma trigliserit düzeyine etkili olurken, tek başına vitamin A ilavesi bu düzeyi artırdı. Plazmadaki fosfor seviyesi, rasyona çinko ilavesi ve çinkonun vitamin A ile etkileşimi sonucunda değişti. Plazma çinkosu ile yumurta verimi arasında pozitif bir ilişki gözlendiğinden, yumurta verimi ya da tavuk performansının ölçümü için plazma çinko düzeyinin bir gösterge olabileceği öne sürüldü.

Anahtar Sözcükler: çinko, vitamin A, yumurta verimi.

Introduction

Vitamin A increased the accumulation and transport of zinc in chick ileal mucosa and the stimulation of zinc absorption, which may be related to a specific carrier-vitamin A dependent zinc binding protein. This protein was shown to be highly specific for zinc ions (1). Zinc is

necessary for the normal mobilisation of vitamin A from the liver into plasma, and therefore to maintain the normal plasma vitamin A concentrations (2). The absorption, transport and utilisation of vitamin A are also influenced by zinc status (3, 4).

Plasma zinc seems to fall only when dietary intake is so low that homeostasis cannot be established without the use of some zinc from the exchangeable pool, of which plasma zinc is a component. Thus, plasma zinc is a valid, useful indicator of the size of exchangeable pool zinc; a reduction in plasma zinc reflects a loss of zinc from the bones and liver and an increased risk of the development of metabolic and clinical signs of zinc deficiency (5).

The yolk precursor molecule vitellogenin transfers trace elements to the maturing oocyte and, within the yolk, lipovitellin and phosphitin serve as metal storage sites (6). Lipovitellin binds more than 90% of the yolk zinc (7). The levels of plasma zinc and lipoprotein organic phosphorus in mature laying hens were reported to be higher than those of immature females and mature male birds due to plasma vitellogenin (8). It was also suggested that the measurement of plasma zinc provides a simple and accurate technique for the estimation of the reproductive status in domestic fowl by Mitchell and Carlisle (8).

Egg production rate was reduced by vitamin A deficient diets (9,10). Supplementing 0, 10, 20 and 40 mg zinc/kg of diet did not change the rate of laying (11) except 20 g zinc/kg given for moulting (12).

Lower activities of the plasma lipoprotein lipase and triglyceride lipase and higher plasma triglyceride values were found in the layer state than in the brooder and grower states (8,13,14).

Since there is not much information about the effects of the supplementation of vitamin A and zinc together in laying hens, except the independent effects of zinc and vitamin A or the feeding of toxic levels of these, this study was undertaken to investigate the effects of various levels of both zinc and vitamin A supplementation on the rate of laying, the plasma and yolk concentration of zinc, the levels of plasma triglyceride and phosphorus and to test plasma zinc is an accurate technique for egg production in layers.

Materials and Methods

One hundred and thirty Hisex Brown laying hens were obtained from the Department of Animal Husbandry, Selçuk University, Konya, Turkey. The hens were divided into two groups. One group of hens consumed no vitamin A supplemented diet (0 IU/kg, Group A), while another group of hens consumed 10.000 IU vitamin A/kg (Roche Müstahzarları A.Ş., İstanbul, Turkey) supplemented diet (Group B). Each group of hens was further divided into five subgroups (13 hens in each subgroup) fed zinc, as zinc oxide (Doğa İlaç, İstanbul, Turkey), to provide the supplementation of 0, 25, 50, 100, 200 mg zinc/kg of diet (A1-A5, B1-B5). Each hen was put into individual stainless steel cages after being weighed. The lighting pattern was 16 hours light and the temperature was 22±0.5°C. Hens received the experimental diets and water *ad libitum* for 12 weeks (Table 1).

Egg yields were recorded daily, while egg weights and feed consumption were determined monthly. Blood samples were collected via cardiac puncture from the hens following an overnight fast at the end of the trial.

Table 1. Composition of the basal diet.

Ingredients	g kg^{-1}
Corn	500.0
Soybean meal	253.5
Wheat	140.0
Vegetable oil	10.0
Limestone	80.0
Dicalcium phosphate	10.0
Salt	3.0
Vitamin mix[1,2]	2.5
Mineral mix[3,4]	1.0
Chemical Analysis	
Dry matter	902.80
Ash	84.40
Crude fiber	45.30
Crude protein	174.70
Ether extract	54.20

[1]: Vitamin mixtures provide (mg/kg diet except were noted): dl-α - tocopheryl acetate, 20; cholecalcipherol 30, µg; thiamine, 3; riboflavin, 7; menadione, 5; niacin, 20; vit B_6, 5; vit B_{12} ,0.015; folic acid, 1; ascorbic acid, 50; choline chloride, 200;

[2]: Vitamin A level was 4750 IU/kg of diet.

[3]: Mineral mixtures provide (mg/kg diet): manganese, 80; iron, 60;copper, 5; cobalt, 0.2; iodine, 1; calcium carbonate, 20; D-biotine, 0.05.

[4]: Zn in the diet and drinking water were 46 mg/kg and 1.11 µg/ml, respectively.

The blood samples were transferred into heparinised tubes. Plasmas were stored frozen at –20°C for later analysis. Plasma triglyceride and inorganic phosphorus were analysed by UV Spectrophotometer using commercial kits (Diasys, Diagnostic Systems, Germany).

One millilitre of plasma was diluted with 4 mL of 1% HCl in distilled water (15) for zinc analysis. After the eggs were hard boiled, the yolks were separated and homogenised in petri dishes. One gram of egg yolk and diet samples was put into porcelain crucibles and ashed for 18 hours at 500°C in a muffle furnace. All ash residues were dissolved in HCl and diluted to 100 mL with distilled water (16). The zinc concentration of the samples was determined by an atomic absorption spectrophotometer (Buck Scientific 200A) at 213.9 nm.

Data were evaluated statistically by an analysis of variance with post hoc separation of treatment means by Duncan's multiple range test. In each case, a p-value less than 0.05 was considered significant. Egg production data were converted by arc sine transformation before being analysed and presented as percentages. These analyses were accomplished by using a statistical analysis system configured for a computer (17).

Results

Adding vitamin A and/or zinc to the hen's diet resulted in no significant differences in body weight, egg production, egg weight, feed intake and feed conversion among the groups of hens receiving experimental diets (Table 2).

Plasma zinc concentration was affected only by dietary zinc supplementation (Table 3). The highest plasma zinc level was 4.39 µg/ml in the group fed 100 mg zinc/kg in the diet, and was very close to the plasma zinc level of the control group. The group fed 200 mg zinc/kg of diet had significantly lower plasma zinc concentrations than those of the control and 100 mg zinc/kg supplemented groups (Table 4).

Vitamin A and zinc interaction affected the plasma triglyceride level (Table 3), and adding vitamin A was very effective on this significant interaction for plasma triglycerides (Figure 1). When analysis of variance were performed within each of the vitamin A groups, it was found that the triglyceride concentration of group A_5 (suppl. no vit A and 200 mg zinc/kg) was higher than that of groups A_3 and A_4. Group A_4 (suppl. no vit A and 100 mg zinc/kg) had a lower triglyceride concentration than

Table 2. The results of the performance parameters of the experimental groups[1,2].

Supplements																
Vitamin A[3] IU/kg		Zinc[4] mg/kg		Initial body weight, kg		Final Body weight, kg		Egg production,%		Egg weight, g		Feed intake, g day^{-1}		Feed conversion, kg feed kg egg^{-1}		Specific gravity, g cm^{3} $^{-1}$
	Group A		n		n		n		n		n		n		n	
0	A1	0	13	1.85±0.05	11	1.71±0.06	9	80.77±2.57	9	62.13±2.18	11	119.0±1.77	9	2.35±0.14	9	1.07±0.0
	A2	25	13	1.91±0.04	13	1.65±0.04	13	76.30±2.13	13	65.00±1.92	13	121.4±1.05	13	2.46±0.08	13	1.08±0.0
	A3	50	13	1.77±0.03	13	1.63±0.04	12	80.33±1.81	12	62.49±0.54	13	122.1±2.67	12	2.53±0.14	12	1.08±0.0
	A4	100	13	1.87±0.04	12	1.85±0.05	12	80.25±2.19	12	65.33±1.86	12	123.6±1.70	12	2.21±0.08	12	1.08±0.0
	A5	200	13	1.84±0.03	12	1.80±0.06	10	77.70±3.71	10	63.82±1.93	12	124.0±2.04	10	2.47±0.15	10	1.07±0.0
	Group B															
10.000	B1	0	13	1.83±0.03	11	1.74±0.04	10	81.20±2.28	10	63.49±1.40	11	120.9±1.19	10	2.51±0.17	10	1.07±0.0
	B2	25	13	1.80±0.03	12	1.76±0.05	11	82.90±1.99	11	64.72±1.12	12	121.6±1.38	11	2.66±0.37	11	1.08±0.0
	B3	50	13	1.82±0.03	13	1.76±0.06	13	80.38±1.26	13	66.66±1.70	13	119.5±1.83	13	2.26±0.05	13	1.08±0.0
	B4	100	13	1.81±0.03	12	1.74±0.06	11	79.18±2.06	11	64.14±1.44	12	118.9±1.37	11	2.30±0.10	11	1.07±0.0
	B5	200	13	1.84±0.03	13	1.71±0.05	12	79.16±1.88	12	62.88±1.56	13	118.8±1.73	12	2.39±0.08	12	1.07±0.0

[1] Means±SEM.

[2] No significant differences were determined among groups A and B.

[3] Supplemented as retinyl acetate.

[4] Supplemented as ZnO

that of A_2 and A_5. Vitamin A and zinc supplementation lowered the plasma triglyceride level in group B_5 when compared to the level of the control group (Table 3). Supplementing vitamin A increased the plasma triglyceride level from 10.98 mg/ml to 14.85 mg/ml (Table 5).

The effect of zinc supplementation alone and also zinc and vitamin A interaction was significant on the concentration of plasma phosphorus. Not adding vitamin A was much more effective on the interaction for the plasma phosphorus level (Figure 2). However, the 200 mg zinc/kg supplementation had higher plasma phosphorus levels than other groups except the control group and the 50 mg zinc/kg supplemented group (Table 4). Vitamin A supplementation in the group without zinc supplement (B_1) and the 200 mg zinc/kg supplementation for group A had increased levels of plasma phosphorus (Table 3).

Yolk zinc was only affected by dietary zinc supplementation. The level of yolk zinc of the group fed

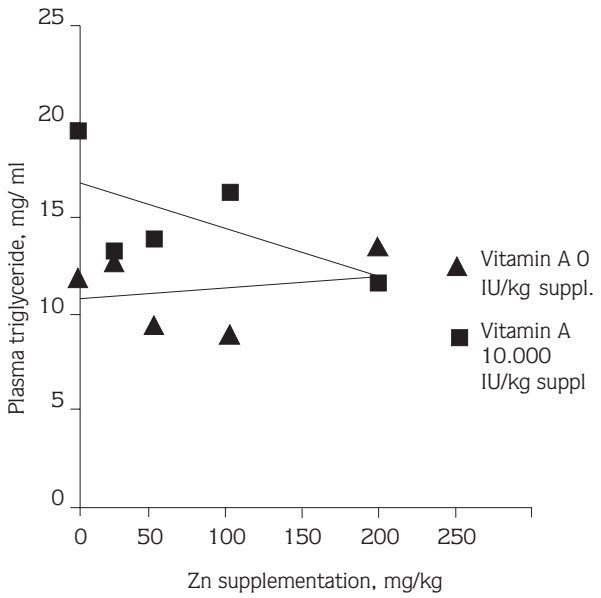

Figure 1. Effect of vitamin A- zinc interaction on plasma triglyceride levels in laying hens.

Table 3. Effect of dietary vitamin A and zinc on plasma zinc, triglyceride, phosphorus and yolk zinc of laying hens[1,2].

Supplements										
Vitamin A[3]		Zinc[4]	Plasma zinc		Plasma triglyceride		Plasma phosphorus		Yolk zinc	
IU/kg		mg/kg	µg/ml		mg/ml		mg/dl		µg/g	
	Group A		n		n		n		n	
0	A1	0	10	4.20±0.33	7	11.74±1.30^{abc}	7	4.46±0.42^{b}	8	38.36±1.18
	A2	25	10	3.59±0.19	8	12.65±1.32^{ab}	13	4.60±0.35^{b}	7	30.35±2.73
	A3	50	11	3.71±0.25	9	9.37±1.22^{bc}	8	6.10±0.61^{ab}	10	39.48±3.29
	A4	100	12	4.29±0.30	10	8.86±0.87^{c}	10	4.46±0.45^{b}	7	28.87±2.19
	A5	200	12	3.71±0.28	7	13.40±1.10^{a}	10	6.83±0.99^{a}	9	28.89±3.86
	Group B									
10.000	B1	0	10	4.27±0.29	7	19.62±2.76^{a}	8	7.30±0.55^{a}	9	30.91±3.73
	B2	25	9	3.82±0.33	7	13.22±2.13^{ab}	8	4.68±0.42^{b}	8	29.97±2.36
	B3	50	11	3.71±0.26	7	13.87±2.08^{ab}	9	5.74±0.28^{b}	6	40.04±2.64
	B4	100	11	4.51±0.34	7	16.36±3.08^{ab}	10	5.51±0.44^{b}	6	26.16±2.73
	B5	200	10	3.47±0.19	8	11.62±1.12^{b}	9	5.96±0.63^{ab}	8	33.33±3.71
Analysis of Variance										
Vitamin A				NS		S		NS		NS
Zinc				S[5]		NS		S		S
Vitamin A X Zinc				NS		S		S		NS

[1] Means ± SEM.
[2] In each column within each group, nonmatching superscripts denote significant differences at $p< .05$.
[3] Supplemented as retinyl acetate.
[4] Supplemented as ZnO.
[5] Significant at $p< .05$.

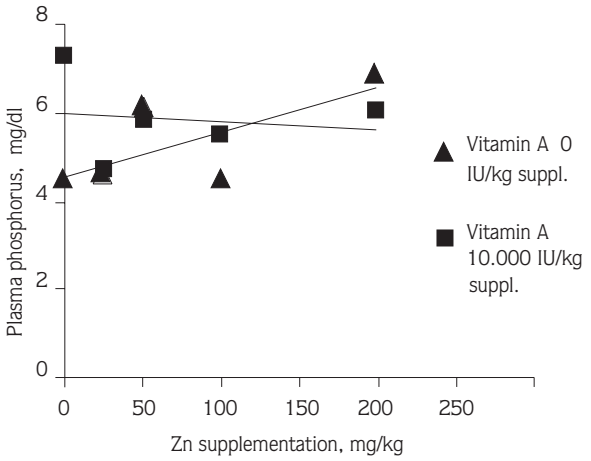

Figure 2. Effect of vitamin A- zinc interaction on plasma phosphorus levels in laying hens

50 mg zinc/kg was close to the level of the control group, but was significantly higher than that of other zinc supplemented groups (Table 4).

Discussion

Vitamin A should be supplemented to layer diets so as to compensate for alterations in the vitamin levels of feed ingredients, oxidation loss and those demands due to feed consumption, stress, genotype and management (18). Zinc deficiency lowers the rate of growth, feed efficiency and egg production (4). Although Richter (19) reported that feed consumption, laying performance, egg weight and feed efficiency are dependent on vitamin A supply, some researchers found that supplementing different amounts of vitamin A (20, 21) and zinc (11, 22) to layer diets did not alter the performance parameters of laying hens. In the present study, layer hens fed experimental diets showed no difference in feed consumption, egg yield, egg weight and feed efficiency.

Some evidence shows that a diminished supply of dietary zinc may increase zinc absorption in the uptake phase. It has been suggested that two mechanisms of zinc

Table 4. Effect of adding zinc on plasma zinc, triglyceride, phosphorus and yolk zinc of laying hens.[1,2]

Supplement								
Zinc[3] mg/kg	n	Plasma zinc µg/ml	n	Plasma triglyceride mg/ml	n	Plasma phosphorus mg/dl	n	Yolk zinc µg/g
0	20	4.23±0.21^{ab}	14	15.68±1.82	15	5.97±0.51^{ab}	17	34.42±2.20^{ab}
25	19	3.70±0.18^{bc}	15	12.91±1.17	21	4.63±0.26^{c}	15	30.15±1.72^{b}
50	22	3.71±0.17^{bc}	16	11.34±1.24	17	5.91±0.31^{ab}	16	36.69±2.21^{a}
100	23	4.39±0.22^{a}	17	11.95±1.60	20	4.98±0.33^{bc}	13	27.62±1.70^{b}
200	22	3.60±0.82^{c}	15	12.45±0.80	19	6.42±0.59^{a}	17	30.98±2.66^{b}

[1] Means ± SEM.
[2] In each column, nonmatching superscripts denote significant differences at p<.05.
[3] Supplemented as ZnO

Table 5. Effect of adding vitamin A on plasma zinc, triglyceride, phosphorus and yolk zinc of laying hens.[1,2]

Supplement								
Vitamin A[2] IU/kg	n	Plasma zinc µg/ml	n	Plasma triglyceride mg/ml	n	Plasma phosphorus mg/dl	n	Yolk zinc µg/g
0	55	3.90±0.13	41	10.98±0.57^{b,3}	48	5.27±0.29	41	33.57±1.48
10.000	51	3.96±0.14	36	14.85±1.07^{a}	44	5.83±0.25	37	31.94±1.54

[1] Means ± SEM.
[2] Supplemented as retinyl acetate.
[3] Significant at p<.001

absorption exist. One of these may be induced by low dietary zinc levels. Because altered brush border membrane transport may account for a larger segment of the zinc absorbed at lower luminal zinc concentrations in rats fed zinc deficient diets, the total amount of zinc absorbed when dietary zinc is low may be close to that observed in higher dietary zinc intakes. At high zinc concentrations, the membranes may become leaky and allow zinc to enter the cell and bind nonspecifically to cell proteins and other ligands (23).

Johnson and Greger (24) found that the increased consumption of zinc resulted in the decreased apparent absorption of zinc, increased losses of endogenous zinc in faeces and the decreased true absorption of zinc. Stahl et al. (25) determined that chicks receiving a control diet (37 mg zinc/kg) excreted significantly less of the administered dose of ^{65}Zn than chicks given moderate (103 mg zinc/kg) or excess (2183 mg zinc/kg) amounts of zinc, and that chicks given more than 100 mg zinc/kg of the diet accumulated zinc in their tissues.

In this research, the levels of plasma zinc ranged from 3.47µg/ml to 4.51µg/ml which were in conformity with other reports on domestic fowl (8) and turkey hen (26). The level of plasma zinc in the control group was very close to the level of those hen's fed 100 mg zinc/kg, while plasma zinc declined significantly after the feeding of more than 100 mg zinc/kg. This data may indicate that the plasma zinc level was controlled by the homeostatic mechanism with regards absorption, excretion or accumulation in tissues due to the level of dietary zinc.

Either the absence or excess of vitamin A in the diet increased the triglyceride content in the liver, and it is suggested that the levels of vitamin A have some influences upon the metabolic processes of liver triglyceride (27), and that the daily feeding of vitamin A supplement increased serum triglycerides (28). The rise in the levels of plasma triglyceride was determined due to a decrease in the activities of lipoprotein lipase and triglyceride lipase in laying turkeys by Kelly et al. (13). The values estimated for plasma triglyceride in the present study ranged from 8.86 mg/ml to 19.62 mg/ml, and were very close to results reported by others (8,13), while adding vitamin A significantly increased plasma triglyceride. Although plasma phosphorus levels were shown to decrease in turkeys through vitamin A supplementation at 16 000 IU/kg (29), there were no differences noted in this research following the addition of vitamin A.

Williams et al. (30) reported that excess dietary zinc (20 g zinc/kg of diet) was accumulated in the tissues, and yolk zinc was increased threefold. Feeding 10-30 g zinc/kg resulted in the highest yolk zinc in the group fed the lowest (10 g zinc/kg) dietary zinc (31). Adding zinc affected yolk zinc levels, and the estimated values of yolk zinc ranged from 27.62 µg/g to 36.69 µg/g and these levels were similar to the results of the control group determined by Decuypere et al. (31).

Mitchell and Carlisle (8) reported that laying birds exhibited much higher levels of zinc than did males or immature female birds, consistent with the presence of vitellogenin in the plasma, and plasma zinc in female birds of different ages confirms the applicability of this parameter as an index of circulating vitellogenin in relation to reproductive status. In the current research, the positive correlation between plasma zinc and egg production ($r= 0.279$, $p< 0.01$) was observed and suggests that plasma zinc concentrations were affected by egg production (8).

In conclusion, the supplementation of zinc (up to 200 mg/kg) and vitamin A (0-10.000 IU/kg) in the diet of laying hens for 12 weeks had no effect on performance parameters. While adding zinc affected plasma and yolk zinc concentration and plasma phosphorus, adding vitamin A only increased plasma triglyceride levels, which all were in the normal range. So if a diet based on corn, soybean meal and wheat contains ~ 5000 IU vitamin A and ~ 50 mg zinc/kg, it might meet the demand for zinc and vitamin A under normal conditions for laying hens. As suggested by Mitchell and Carlisle (8), the positive correlation between plasma zinc and egg production demonstrates that plasma zinc may be an indicator for the estimation of egg yield in layers.

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